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☑ 1. 6555389 . 23 Dec 99; 29 Apr 03. Sample evaporative control. Ullman; Edwin F., et al. 436/514; 422/101 422/55 422/58 422/68.1 422/82.01 435/287.1 435/288.4 435/288.5 436/149 436/150 436/151 436/518 436/535. G01N033/558.

☑ 2. 6413780 . 11 Oct 99; 02 Jul 02. Structure and method for performing a determination of an item of interest in a sample. Bach; Mark C., et al. 436/48; 198/347.3 198/347.4 422/63 422/64 422/65 422/68.1 422/82.05 435/287.1 436/164 436/172 436/43 436/47 436/49. G01N035/02.

☑ 3. 6319469 . 18 Dec 96; 20 Nov 01. Devices and methods for using centripetal acceleration to drive fluid movement in a microfluidics system. Mian; Alec, et al. 422/64; 422/63 422/67 422/72 436/45. G01N021/07.

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Previous Page Next Page

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L8: Entry 4 of 5 File: USPT Jan 2, 2001

DOCUMENT-IDENTIFIER: US 6168948 B1
TITLE: Miniaturized genetic analysis systems and methods

Brief Summary Text (7):
A number of researchers have attempted to employ these microfabrication techniques in the miniaturization of some of the processes involved in genetic analysis in particular. For example, published PCT Application No. WO 94/05414, to Northrup and White, incorporated herein by reference in its entirety for all purposes, reports an integrated micro-PCR apparatus for collection and amplification of nucleic acids from a specimen. However, there remains a need for an apparatus which combines the various processing and analytical operations involved in nucleic acid analysis. The present invention meets these and other needs.

Drawing Description Text (10):
FIG. 9A is a gel showing a time course of an RNA fragmentation reaction. FIG. 9B is a gel showing a comparison of the product of an in vitro transcription reaction in a microchamber vs. a control (test tube). FIG. 9C is a comparison of the PCR product produced in a PCR thermal cycler and that produced by a microreactor.

Drawing Description Text (15):
FIG. 14 shows a demonstration of integrated reactions in a microfabricated polycarbonate device. FIG. 14A shows the layout of the device including the thermal configuration of the device. FIG. 14B shows the results of PCR amplification and subsequent in vitro transcription within the chambers of the device.

Drawing Description Text (22):
FIG. 20 is a schematic representation of a miniaturized device for performing rapid thermal cycling reactions, such as PCR or RT-PCR.

Drawing Description Text (60):
FIG. 51 illustrates a top view of a polycarbonate cartridge for simultaneously performing preparative reactions including PCR, fragmentation, and labeling on four separate samples. PCR reactions.

Detailed Description Text (5):
To carry out their primary functions, one embodiment of the devices of the invention will typically incorporate a plurality of distinct reaction chambers for carrying out the sample acquisition, preparation and analysis operations. In particular, a sample to be analyzed is introduced into the device whereupon it will be delivered to one of these distinct reaction chambers which are designed for carrying out a variety of reactions as a prelude to analysis of the sample. These preparative reactions generally include, e.g., sample extraction, PCR amplification, nucleic acid fragmentation and labeling, extension reactions, transcription reactions and the like.

Detailed Description Text (25):
Following sample collection and nucleic acid extraction, the nucleic acid portion of the sample is typically subjected to one or more preparative reactions. These preparative reactions include in vitro transcription, labeling, fragmentation, amplification and other reactions. Nucleic acid amplification increases the number of copies of the target nucleic acid sequence of interest. A variety of amplification methods are suitable for use in the methods and device of the present invention, including for example, the polymerase chain reaction method or (PCR), the ligase chain reaction (LCR), self sustained sequence replication (3SR), and nucleic acid based sequence amplification (NASBA).

Detailed Description Text (27):
In particularly preferred aspects, the amplification step is carried out using PCR techniques that are well known in the art. See PCR Protocols: A Guide to Methods and Applications (Innis, M., Gelfand, D., Sninsky, J. and White, T., eds.) Academic Press (1990), incorporated herein by reference in its entirety for all purposes. PCR amplification generally involves the use of one strand of the target nucleic acid sequence as a template for producing a large number of complements to that sequence. Generally, two primer sequences complementary to different ends of a segment of the complementary strands of the target sequence hybridize with their respective strands of the target sequence, and in the presence of polymerase enzymes and deoxy-nucleoside triphosphates, the primers are extended along the target sequence. The extensions are melted from the target sequence and the process is repeated, this time with the additional copies of the target sequence synthesized in the preceding steps. PCR amplification typically involves repeated cycles of denaturation, hybridization and extension reactions to produce sufficient amounts of the target nucleic acid. The first step of each cycle of the PCR involves the separation of the nucleic acid duplex formed by the primer extension. Once the strands are separated, the next step in PCR involves hybridizing the separated strands with primers that flank the target sequence. The primers are then extended to form complementary copies of the target strands. For successful PCR amplification, the primers are designed so that the position at which each primer hybridizes along a duplex sequence is such that an extension product synthesized from one primer, when separated from the template (complement), serves as a template for the extension of the other primer. The cycle of denaturation, hybridization, and extension is repeated as many times as necessary to obtain the desired amount of amplified nucleic acid.

Detailed Description Text (28):
In PCR methods, strand separation is normally achieved by heating the reaction to a sufficiently high temperature for a sufficient time to cause the denaturation of the duplex but not to cause an irreversible denaturation of the polymerase enzyme (see U.S. Pat. No. 4,965,188, incorporated herein by reference). Typical heat denaturation involves temperatures ranging from about 80.degree. C. to 105.degree. C. for times ranging from seconds to minutes. Strand separation, however, can be accomplished by any suitable denaturing method including physical, chemical, or enzymatic means. Strand separation may be induced by a helicase, for example, or an enzyme capable of exhibiting helicase activity. For example, the enzyme RecA has helicase activity in the presence of ATP. The reaction conditions suitable for strand separation by helicases are known in the art (see Kuhn Hoffman-Berling, 1978, CSH-Quantitative Biology, 43:63-67; and Radding, 1982, Ann. Rev. Genetics 16:405-436, each of which is incorporated herein by reference). Other embodiments may achieve strand separation by application of electric fields across the sample. For example, Published PCT Application Nos. WO 92/04470 and WO 95/25177, incorporated herein by reference, describe electrochemical methods of denaturing double stranded DNA by application of an electric field to a sample containing the DNA. Structures for carrying out this electrochemical denaturation include a working electrode, counter electrode and reference electrode arranged in a potentiostat arrangement across a reaction chamber (See, Published PCT Application Nos. WO 92/04470 and WO 95/25177, each of which is incorporated herein by reference for all purposes). Such devices may be readily miniaturized for incorporation into the devices of the present invention utilizing the microfabrication techniques described herein.

Detailed Description Text (29):
Template-dependent extension of primers in PCR is catalyzed by a polymerizing agent in the presence of adequate amounts of at least 4 deoxyribonucleotide triphosphates (typically selected from DATP, dGTP, dCTP, dUTP and dTTP) in a reaction medium which comprises the appropriate salts, metal cations, and pH buffering system. Reaction components and conditions are well known in the art (See PCR Protocols: A Guide to Methods and Applications (Innis, M., Gelfand, D., Sninsky, J. and White, T., eds.) Academic Press (1990), previously incorporated by reference). Suitable polymerizing agents are enzymes known to catalyze template-dependent DNA synthesis.

Detailed Description Text (30):
Published PCT Application No. WO 94/05414, to Northrup and White, discusses the use of a microPCR chamber which incorporates microheaters and micropumps in the thermal cycling and mixing during the PCR reactions.

Detailed Description Text (31):
The amplification reaction chamber of the device may comprise a sealable opening for the addition of the various amplification reagents. However, in preferred aspects, the amplification chamber will have an effective amount of the various amplification reagents described above, predisposed within the amplification chamber, or within an associated reagent chamber whereby the reagents can be readily transported to the amplification chamber upon initiation of the amplification operation. By "effective amount" is meant a quantity and/or concentration of reagents required to carry out amplification of a targeted nucleic acid sequence. These amounts are readily determined from known PCR protocols. See, e.g., Sambrook, et al. Molecular Cloning: A Laboratory Manual, (2nd ed.) Vols. 1-3, Cold Spring Harbor Laboratory, (1989) and PCR Protocols: A Guide to Methods and Applications (Innis, M., Gelfand, D., Sninsky, J. and White, T., eds.) Academic Press (1990), both of which are incorporated herein by reference for all purposes in their entirety. For those embodiments where the various reagents are predisposed within the amplification or adjacent chamber, it will often be desirable for these reagents to be in lyophilized forms, to provide maximum shelf life of the overall device. Introduction of the liquid sample to the chamber then reconstitutes the reagents in active form, and the particular reactions may be carried out.

Detailed Description Text (32):
In some aspects, the polymerase enzyme may be present within the amplification chamber, coupled to a suitable solid support, or to the walls and surfaces of the amplification chamber. Suitable solid supports include those that are well known in the art, e.g., agarose, cellulose, silica, divinylbenzene, polystyrene, etc. Coupling of enzymes to solid supports has been reported to impart stability to the enzyme in question, which allows for storage of days, weeks or even months without a substantial loss in enzyme activity, and without the necessity of lyophilizing the enzyme. The 94 kd, single subunit DNA polymerase from Thermus aquaticus (or taq polymerase) is particularly suited for the PCR based amplification methods used in the present invention, and is generally commercially available from, e.g., Promega, Inc., Madison, Wis. In particular, monoclonal antibodies are available which bind the enzyme without affecting its polymerase activity. Consequently, covalent attachment of the active polymerase enzyme to a solid support, or the walls of the amplification chamber can be carried out by using the antibody as a linker between the enzyme and the support.

Detailed Description Text (33):
In addition to PCR and IVT reactions, the methods and devices of the present invention are also applicable to a number of other reaction types, e.g., reverse transcription, nick translation, cDNAse generation, and the like.

Detailed Description Text (37):
Labeling may also be carried out by attaching an appropriately labeled (e.g. FICT, or biotin), dNTP to the 3'-end of DNAase fragmented PCR product using terminal deoxy-transferase (TdT).

Detailed Description Text (56):
The hybridization data is next analyzed to determine the presence or absence of a particular sequence within the sample, or by analyzing multiple hybridizations to determine the sequence of the target nucleic acid using the SBH techniques already described.

Detailed Description Text (60):
Microcapillary array electrophoresis generally involves the use of a thin capillary or channel which may or may not be filled with a particular separation medium. Electrophoresis of a sample through the capillary provides a size based separation profile for the sample. The use of microcapillary electrophoresis in size separation of nucleic acids has been reported in, e.g., Woolley and Mathies, Proc. Nat'l Acad. Sci. USA (1994) 91:11348-11352. Microcapillary array electrophoresis generally provides a rapid method for size based sequencing, PCR product analysis and restriction fragment sizing. The high surface to volume ratio of these capillaries allows for the application of higher electric fields across the capillary without substantial thermal variation across the capillary, consequently allowing for more rapid separations. Furthermore, when combined with confocal imaging methods, these

methods provide sensitivity in the range of attomoles, which is comparable to the sensitivity of radioactive sequencing methods.

Detailed Description Text (98):
For PCR amplification methods, denaturation and hybridization cycling will preferably be carried out by repeated heating and cooling of the sample. Accordingly, PCR based amplification chambers will typically include a temperature controller for heating the reaction to carry out the thermal cycling. For example, a heating element or temperature control block may be disposed adjacent the external surface of the amplification chamber thereby transferring heat to the amplification chamber. In this case, preferred devices will include a thin external wall for chambers in which thermal control is desired. This thin wall may be a thin cover element, e.g., polycarbonate sheet, or high temperature tape, i.e. silicone adhesive on Kapton tape (commercially available from, e.g., 3M Corp.). Micro-scale PCR devices have been previously reported. For example, published PCT Application No. WO 94/05414, to Northrup and White reports a miniaturized reaction chamber for use as a PCR chamber, incorporating microheaters, e.g., resistive heaters. The high surface area to volume ratio of the chamber allows for very rapid heating and cooling of the reagents disposed therein. Similarly, U.S. Pat. No. 5,304,487 to Wilding et al., previously incorporated by reference, also discusses the use of a microfabricated PCR device.

Detailed Description Text (99):
In preferred embodiments, the amplification chamber will incorporate a controllable heater disposed within or adjacent to the amplification chamber, for thermal cycling of the sample. Thermal cycling is carried out by varying the current supplied to the heater to achieve the desired temperature for the particular stage of the reaction. Alternatively, thermal cycling for the PCR reaction may be achieved by transferring the fluid sample among a number of different reaction chambers or regions of the same reaction chamber, having different, although constant temperatures, or by flowing the sample through a serpentine channel which travels through a number of varied temperature `zones`. Heating may alternatively be supplied by exposing the amplification chamber to a laser or other light or electromagnetic radiation source.

Detailed Description Text (128):
A number of the operations performed by the various reaction chambers of the device require a controllable temperature. For example, PCR amplification, as described above, requires cycling of the sample among a strand separation temperature, an annealing reaction temperature and an extension reaction temperature. A number of other reactions, including extension, transcription and hybridization reactions are also generally carried out at optimized, controlled temperatures. Temperature control within the device of the invention is generally supplied by thin film resistive heaters which are prepared using methods that are well known in the art. For example, these heaters may be fabricated from thin metal films applied within or adjacent to a reaction chamber using well known methods such as sputtering, controlled vapor deposition and the like. The thin film heater will typically be electrically connected to a power source which delivers a current across the heater. The electrical connections will also be fabricated using methods similar to those described for the heaters.

Detailed Description Text (130):
Temperature controlled reaction chambers will also typically include a miniature temperature sensor for monitoring the temperature of the chamber, and thereby controlling the application of current across the heater. A wide variety of microsensors are available for determining temperatures, including, e.g., thermocouples having a bimetallic junction which produces a temperature dependent electromotive force (EMF), resistance thermometers which include material having an electrical resistance proportional to the temperature of the material, thermistors, IC temperature sensors, quartz thermometers and the like. See, Horowitz and Hill, The Art of Electronics, Cambridge University Press 1994 (2nd Ed. 1994). One heater/sensor design that is particularly suited to the device of the present invention is described in, e.g., U.S. patent application Ser. No. 08/535,875, filed Sep. 28, 1995, and incorporated herein by reference in its entirety for all purposes. Control of reaction parameters within the reaction chamber, e.g., temperature, may be carried out manually, but is preferably controlled via an appropriately programmed computer. In particular, the temperature measured by the temperature sensor and the input for the

power source will typically be interfaced with a computer which is programmed to receive and record this data, i.e., via an analog-digital/digital-analog (AD/DA) converter. The same computer will typically include programming for instructing the delivery of appropriate current for raising and lowering the temperature of the reaction chamber. For example, the computer may be programmed to take the reaction chamber through any number of predetermined time/temperature profiles, e.g., thermal cycling for PCR, and the like. Given the size of the devices of the invention, cooling of the reaction chambers will typically occur through exposure to ambient temperature, however additional cooling elements may be included if desired, e.g., coolant systems, peltier coolers, water baths, etc. Alternatively, thermoelectric coolers can be used to maintain the temperature by being pressed against the thin wall.

Detailed Description Text (131):
In addition to fluid transport and temperature control elements, one or more of the reaction chambers of the device may also incorporate a mixing function. For a number of reaction chambers, mixing may be applied merely by pumping the sample back and forth into and out of a particular reaction chamber. However, in some cases constant mixing within a single reaction/analytical chamber is desired, e.g., PCR amplification reactions and hybridization reactions.

Detailed Description Text (156):
The storage domain is linked to the sample preparation domain, and is used for storage of reagents and mixtures, e.g., PCR mix with FITC-dGTP and dUTP but no template, UNG reaction mix and IVT reaction mix without template. The reaction domain is also linked to the sample preparation domain as well as the storage domain and includes a number of reaction chambers (5), measuring chambers (2) and debubbling chambers (1). Both sample preparation and reaction domains may be addressed by a thermal controller, e.g., heaters or thermoelectric heater/cooler.

Detailed Description Text (159):
FIG. 13 is a schematic illustration of a device configuration for addressing situations where several reactions are to be carried out under the same thermal conditions, e.g., multiple parallel sample analyses, duplicating multiplex PCR by carrying out several PCR reactions with single primer pairs in parallel followed by recombining them, or cycle sequencing with a variety of primer pairs and/or templates.

Detailed Description Text (170):
In use, a sample such as whole blood is introduced into the chamber 1902 through an inlet channel 1920 under conditions so that the antibodies 1912 bind to the corresponding cell receptors within the sample. The chamber is washed while the cells remain attached, and then the cells are lysed by the introduction of a lysing agent, such as chaotropic salt. Alternatively, the cells may be lysed by heating them in a hypotonic solution, or adding an enzymatic lysing agent such as protenese K. The lysed cells are then drawn from chamber 1902 through inlet channel 1920 or a second outlet channel 1922. Extraction of the total nucleic acid from this lysate is carried out in a subsequent chamber, as discussed above in reference to FIG. 15. Alternatively, the nucleic acid extraction and subsequent amplification (i.e., PCR) may be performed in-situ within chamber 1902. Temperatures for affinity, washing, and lysis are controlled using a heating element (not shown) pressed against one wall of the cartridge 1901.

Detailed Description Text (206):
Referring again to FIG. 38, an alternative linker structure 4500 takes advantage of the above described vent behavior. As shown, linker structure 4500 comprises a vent 4502 coupled to first and second valves 4504, 4506 so as to form a T-shaped linker structure. This T-shaped linker structure can be used to link two fluid plugs. For example, a first fluid plug 4508 is introduced through valve 4504 to vent 4502, as shown in FIG. 39A. The vent is then cleared by blowing air therethrough, which expels an excess part of fluid 4508 through the second valve 4506 (FIG. 39B). A second fluid plug 4510 is then introduced through the second valve 4506 to vent 4502 to link the first and second fluid plugs 4508, 4510 (FIG. 39C). This process has been demonstrated hundreds of times without failures using mock PCR mixes, real reagents, and solutions with up to five times the amount of Tween-20.

Detailed Description Text (223):
Sample preparation generally requires amplification, usually involving a thermal cycling reaction such as PCR or RT-PCR. The time consumed for this reaction can be significant, as shown below in Table 1. The first line shows some typical parameters for PCR carried out in a PE2400 machine, while the second line shows the same reaction with 10 times the thermal ramp rate (10.degree. C./sec versus 1.degree. C./sec) and reduced denature and anneal times. As shown in the table, significant reduction in processing time is provided by rapid thermal cycling. Also, the temperature of the reaction chamber should be uniform throughout the reactant mixture to maintain product specificity.

Detailed Description Text (235):
In use with the present invention, each heater within the array 2302 is used to control the temperature of an individual reaction chamber within the genetic analysis system. The thermoelectric cooler 2306 functions to provide rapid cooling to all of the reaction chambers. During a rapid thermal reaction, such as PCR, the cooler 2306 is ON throughout the entire reaction. The heater is turned ON to maintain the reaction temperature. When the reaction temperature should be lowered, the heater is turn OFF, and the cooler 2306 rapidly decreases the temperature within the chamber.

Detailed Description Text (237):
FIGS. 24 and 25 are schematic illustrations of hermetically sealed microfluidic systems for genetic analysis according to the present invention. In general, PCR reactions are extremely sensitive, but produce a high concentration of DNA product. This combination creates the danger of cross-contamination leading to erroneous results. A disposable cartridge may, for example, contaminate an instrument through PCR-product aerosols that could find their way into cartridges used in subsequent tests.

Detailed Description Text (249):
In another embodiment, motion of the membrane is provided using forces other than pneumatic (e.g., electrostatic, magnetic, or piezoelectric). For example, plate 4925 is metallic and a moving magnetic field moves the plate.

Detailed Description Text (270):
The precise thermal control provided by the device is especially important for (1) denaturation of DNA, particularly as a cycle in PCR, (2) annealing of DNA templates with primers, particularly with PCR, (3) heat denaturization of enzymes, and (4) lysing cells. Advantages of flow-through thermal treatment device 5550 include (1) coverslip 5530, when made of glass, allows easy observation of fluidics, (2) the high thermal conductivity of the silicon ensures that the fluids in chambers 5510 are generally at a uniform temperature, (3) the liquid volume of the device is minimized, (4) the silicon construction the integration of heating, sensing, and control functions.

Detailed Description Text (300):
In a first embodiment, comprising a magnetic device, the emulsion is either an oil based ferrofluid, or coated paramagnetic beads in silicon oil, fluorinert, or mineral oil. The magnetic field is modulated at the valve location by using a coil or by moving the magnet relative to the valve location. The magnetic field causes alignment and linking of the magnetic beads which increases the fluid viscosity and interrupts flow.

Detailed Description Text (309):
In another aspect of the invention, as shown in FIG. 51, a polycarbonate cartridge 6200 for performing PCR reactions is provided. When operating with associated instrumentation under computer control, the cartridge is adapted to simultaneously perform the following on four different samples: (1) store DNAse/calf alkaline phosphatase (CIAP) reagent mix (at 4.degree. C.), (2) store TdT reagent Mix (4.degree. C.), (3) carry out P450 multiplex PCR, (4) store sample of PCR product, (5) join and mix PCR product with DNAse/CIAP mix, (6) incubate mixture, (7) store sample of reaction product, (8) join and mix reaction product with TdT reagent mix, and (9) incubate mixture.

Detailed Description Text (318):

In another embodiment of the present invention, PCR reactions are enhanced when carried out in reaction chambers fabricated from polycarbonate plate, and coated with silicon and parylene, as follows.

Detailed Description Text (320):
Using such a polycarbonate cartridge coated with silicone and parylene, respectively, a PCR reaction was carried out for Cyp450 multiplex PCR comprising: an initial departure step of 95.degree. C. for 3 minutes, 45 cycles of 95.degree. C. for 45 seconds, 65.degree. C. for 25 seconds and 72.degree. C. for 35 seconds. The extension step of 72.degree. C. was increased by one second after each cycle. The ramping time from annealing (65.degree. C.) to extension (72.degree. C.) was set at 5% for about 40 seconds. Agarose gel (2%) electrophoresis was used to separate DNA bands. The signals were recorded after staining the gel with ethidium bromide. The yield of the cartridge were found to improve significantly as compared to that carried out in PCR in standard format.

Detailed Description Text (328):
In other embodiments of the present invention, DNA polymerization has been carried out with the microfluidic devices described above. Examples of such polymerization include isothermal amplification (NASBA, 3SR, etc), PCR amplification (deep vent, amplitaq gold, taq) and cycle sequencing amplification (with labeled dideoxy terminators, or with labeled primers (e.g., energy transfer dyes). In addition, DNA modification, such as terminal deoxy-transferase (TdT), ligation (including chimeric ligation with RNA) and alkaline phosphatase (removes free 5'OH). Other DNA applications includes DNA fragmentation, such as double stranded DNA (DNAase or restriction endonucleases) or single stranded DNA (nuclease S1) and peptide manipulation, such as in vitro translation and protease digestion.

Detailed Description Text (336):
In separate experiments, HIV cloned DNA was spiked into either horse blood or a suspension of murine plasmacytoma fully differentiated B-cells derived from BALBc mice. Guanidine isothiocyanate was added to a concentration of 4 M, to lyse the material. In separate experiments, the lysate was passed through a cartridge containing glass wool (20 .mu.l), a cartridge with soda glass walls (20 .mu.l), and a glass tube. After 30 minutes at room temperature, the remaining lysate was washed away with several volumes of ethanol:water (1:1) and the captured DNA was eluted at 60.degree. C. using 1.times. TBE. The yield of eluted DNA was measured using ethidum bromide staining on an agarose gel, and purity was tested by using the eluted material as a template for a PCR reaction. Elution yields ranged from 10% to 25% and PCR yields ranged from 90 to 100% as compared to controls using pure template.

Detailed Description Text (342):
Transcription reactions performed in the micro-reactor achieved a 70% yield as compared to conventional methods, e.g., same volume in microfuge tube and water bath or PCR thermal cycler. A comparison of in vitro transcription reaction products using a microchamber versus a larger scale control are shown in FIG. 9B.

Detailed Description Text (344):
PCR Amplification in Miniaturized System

Detailed Description Text (345):
The miniature polymeric reaction chamber similar to the one described in Example 2 was used for carrying out PCR amplification. In particular, the chamber was fabricated from a planar piece of polycarbonate 4 mm thick, and having a cavity measuring 500 .mu.m deep machined into its surface. A second planar polycarbonate piece was welded over the cavity. This second piece was only 250 .mu.m thick. Thermal control was supplied by applying a peltier heater against the thinner second wall of the cavity.

Detailed Description Text (346):
Amplification of a target nucleic acid was performed with Perkin-Elmer GeneAmp.RTM. PCR kit. The reaction chamber was cycled for 20 seconds at 94.degree. C. (denaturing), 40 seconds at 65.degree. C. (annealing) and 50 seconds at 72.degree. C. (extension). Amplification of approximately 10.sup.9 was shown after 35 cycles. FIG. 9C shows production of amplified product in the microchamber as compared to a control using a typical PCR thermal cycler.

Detailed Description Text (349):
A microfabricated polycarbonate device was manufactured having the structure shown in FIG. 14A. The device included three discrete vented chambers. Two of the chambers (top and middle) were thermally isolated from the PCR chamber (bottom) to prevent any denaturation of the RNA polymerase used in IVT reactions at PCR temperatures. Thermal isolation was accomplished by fabricating the chambers more than 10 mm apart in a thin polycarbonate substrate and controlling the temperatures in each region through the use of thermoelectric temperature controllers, e.g., peltier devices.

Detailed Description Text (350):
The reactor device dimensions were as follows: channels were 250 .mu.m wide by 125 .mu.m deep; the three reaction chambers were 1.5 mm wide by 13 mm in length by 125 to 500 .mu.m deep, with the reactor volumes ranging from 2.5 to 10 .mu.l. Briefly, PCR was carried out by introducing 0.3 units of Taq polymerase, 0.2 mM dNTPs, 1.5 mM MgCl.sub.2, 0.2 .mu.M primer sequences, approximately 2000 molecules of template sequence and 1.times. Perkin-Elmer PCR buffer into the bottom chamber. The thermal cycling program included (1) an initial denaturation at 94.degree. C. for 60 seconds, (2) a denaturation step at 94.degree. C. for 20 seconds, (3) an annealing step at 65.degree. C. for 40 seconds, (4) an extension step at 72.degree. C. for 50 seconds, (5) repeated cycling through steps 2-4 35 times, and (6) a final extension step at 72.degree. C. for 60 seconds.

Detailed Description Text (351):
Following PCR, 0.2 .mu.l of the PCR product was transferred to the IVT chamber (middle) along with 9.8 .mu.l of IVT mixture (2.5 mM ATP, CTP, GTP and 0.5 mM UTP, 0.25 mM Fluorescein-UTP, 8 mM MgCl.sub.2, 50 mM HEPES, 1.times. Promega Transcription Buffer, 10 mM DTT, 1 unit T3 RNA polymerase, 0.5 units RNAguard (Pharmacia)) that had been stored in a storage chamber (top). Fluid transfer was carried out by applying pressure to the vents at the termini of the chambers. IVT was carried out at 37.degree. C. for 60 minutes.

Detailed Description Text (352):
The results of PCR and IVT are shown in FIG. 14B, compared with control experiments, e.g., performed in eppendorf tubes.

Detailed Description Text (367):
1. SYS-01 PCR-through Hybridization

Detailed Description Text (368):
The following reactions were carried out under computer control: PCR.sup.i, measurement, mixing, in-vitro transcription (IVT).sup.ii, fragmentation, target dilution, hybridization, and then washing. This system consisted of a modified target-preparation cartridge (model AFFX16) connected to the hybridization cartridge (model AFFX15) along with a pressurized vessel containing 6.times.SSPE. Temperature and fluid movement were controlled using a computer connected peltier devices, solenoid valves, and cartridge-based diaphragm valves and hydrophobic vents. First, the user injects the PCR mixture with template and the IVT reaction mixture into the cartridge. The PCR mix is thermally cycled in the reaction chamber while the IVT mixture is stored in an adjacent chamber held at 3.degree. C. by a second peltier device. After the PCR is completed, part of the mixture is measured in a dosing chamber and the rest expelled. The measured PCR product is combined with the IVT mixture in the debubbling chamber where mixing takes place. This new mixture is transferred back to the reaction chamber where the IVT reaction is carried out at 37.degree. C. generating fluorescently labeled RNA. After 1 hour the temperature is raised to 94.degree. C. for 30 minutes to fragment the RNA. This fragmented product is injected into the hybridization cartridge through tubing addressed by a cartridge-based diaphragm valve. Next, 6.times. SSPE solution enters from a pressurized container, also controlled by a diaphragm valve, and mixes with the labeled RNA target. This liquid is moved into and out of the hybridization chamber for 1 hour. Afterwards, the target mixture is expelled to waste, and several volumes of 6.times. SSPE are injected into the hybridization chamber for washing. Finally, the cartridge is removed for scanning. In this system demonstration, the cumulative PCR and IVT yields were 16% and 40%, respectively, as compared to the control reactions. The GeneChip call rate was 94.4% correct, performance equivalent to that achieved

using standard sample preparation.

Detailed Description Text (370):
The sequence consisting of DNA extraction, PCR, measurement, mixing, in-vitro transcription (IVT), and fragmentation was carried out. This system consists of a modified version of the DNA extraction cartridge described in a previous section, where one of the chambers has a wall made of borosilicate glass. Pressurized vessels containing 50:50 ethanol:water and 1.times.TBE were connected to diaphragm-valve controlled ports on the cartridge for washing and elution, respectively. As in the first system, all thermal control and fluid movement are all accomplished using a computer connected to peltier devices, solenoid valves, cartridge-based diaphragm valves and vents. First, the PCR and IVT mixtures are loaded into storage chambers and maintained at 3.degree. C. Next, a lysate solution with a plasmid containing the HIV sequence (HXB2) in 0.1% BSA and 7 .mu.g/.mu.L hematin and 4M guanidine isothiocyanate is injected into the cartridge and loaded into the extraction chamber. After a 10 minute room-temperature extraction the lysate is automatically ejected to waste. Several volumes of a wash solution (1:1 ethanol; water) are automatically cycled through the extraction chamber and exhausted to waste. The 1.times. TBE is loaded into the chamber and elution carried out at 60.degree. C. for 20 minutes. The eluted template is combined with the PCR mixture in the debubbling chamber, loaded into the reaction chamber and thermally cycled. A portion of the PCR product is combined with the IVT mixture in the debubbling chamber, and this new mixture is shuttled back to the reaction chamber. Incubation at 37.degree. C. for 1 hour generates the labeled RNA target, and the temperature is raised to 94.degree. C. for 30 minutes to fragment the RNA. Finally the target RNA was removed and hybridized manually using conventional methods. For this demonstration, the cumulative IVT yield was 49% as compared with the control, and subsequent hybridization of the fragmented target gave a call rate of 96.5%, equivalent to that achieved using standard methods.

Detailed Description Text (371):
3. SYS-03 PCR through Hybridization in One Cartridge

Detailed Description Text (372):
A cartridge was designed that accommodates a GeneChip array (model AFFX-19) and a similar assay to SYS-01 was performed. The net PCR and IVT yields were 50% and 20%, respectively. The call rate on the HIV chip was 97.1% using the probability method.

Detailed Description Text (374):
Th AFFX-19 cartridge was modified to include a glass-walled extraction chamber. All reactions and processes were carried out: extraction, PCR, in vitro transcription, fragmentation, sample dilution, hybridization, and washing. A simulated blood lysate spiked with HXB2 plasmid, similar to SYS-02 was used as the sample, The net PCR and IVT yields were each approximately 10%. The call rate on the HIV chip was 94.4%.

Detailed Description Text (377):
1. RXN-01 PCR

Detailed Description Text (378):
PCR was performed in ultrasonically welded polycarbonate and polypropylene cartridges. The 10 .mu.L reaction chambers were pretreated with a PCR solution for 30 minutes at room temperature. All reaction yields were equivalent to the control.

Detailed Description Text (381):
I PCR reaction mixture consists of 40 pg/.mu.L of 1.1 kB template DNA, 0.3 units of TAQ polymerase, 1.5 mM MgCl.sub.2, 0.2 mM dNTP's, 0.2 uM primers, and 1.times. Perkin Elmer PCR buffer. Thermal program includes: (1) an initial denature at 94.degree. C. for 60 seconds, (2) a denature at 94.degree. C. for 20 seconds, (3) an anneal at 65.degree. C. for 40 seconds, (4) an extend at 72.degree. C. for 50 seconds, (5) steps 2 through 4 repeated 35 times total, (6) a final extend at 72.degree. C. for 60 seconds.

Detailed Description Paragraph Table (1):
TABLE 1 Effect of Rapid Thermal Cycling on PCR Reaction Time denature ramp anneal ramp extend ramp (sec- (sec- (sec- (sec- (sec- (sec- 35 cycles onds) onds) onds) onds) onds) onds) (minutes) 20 39 20 10 30 29 86 0 3.9 0 1 30 2.9 22

Other Reference Publication (27):
Wooley et al., "Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device," Anal. Chem., 68(23):4081-4086 (1996).

Generate Collection

L8: Entry 3 of 5 File: USPT Nov 20, 2001

DOCUMENT-IDENTIFIER: US 6319469 B1
TITLE: Devices and methods for using centripetal acceleration to drive fluid movement in a microfluidics system

Detailed Description Text (11):
Input and output (entry and exit) ports are components of the microplatforms of the invention that are used for the introduction of removal of a variety of fluid components. Entry ports are provided to allow samples and reagents to be placed on or injected onto the disk; these types of ports are generally located towards the center of the disk. Exit ports are provided to allow air to escape, advantageously into an on-disk "muffler" or "baffle" system, to enable uninhibited fluid movement on the disk. Also included in air handling systems on the disk are air displacement channels, whereby the movement of fluids displaces air through channels that connect to the fluid-containing microchannels retrograde to the direction of movement of the fluid, thereby providing a positive pressure to further motivate movement of the fluid. Exit ports are also provided to allow products to be removed from the disk. Port shape and design vary according specific applications. For example, sample input ports are designed, inter alia, to allow capillary action to efficiently draw the sample into the disk. In addition, ports can be configured to enable automated sample/reagent loading or product removal. Entry and exit ports are most advantageously provided in arrays, whereby multiple samples are applied to the disk using a specifically-designed administration tool. Similar tools are useful designed to effect product removal from the microplatform. Representative arrangements of sample ports, air vents, reagent reservoirs, reaction chambers and microvalves are shown in FIGS. 1A through 1C.

Detailed Description Text (56):
Temperature control elements, particularly heating elements, include heat lamps, direct laser heaters, Peltier heat pumps, resistive heaters, ultrasonication heaters and microwave excitation heaters. Cooling elements include Peltier devices and heat sinks, radiative heat fins and other components to facilitate radiative heat loss. Thermal devices can be applied to the disk as a whole or in specific areas on the disk. The thermal elements can be fabricated directly onto the disk, or can be fabricated independently and integrated onto the disk. Thermal elements can also be positioned external to the disk. The temperature of any particular area on the disk is monitored by resistive temperature devices (RTD), thermistors, liquid crystal birefringence sensors or by infrared interrogation using IR-specific detectors. Temperature at any particular region of the disk can be regulated by feedback control systems. A micro-scale thermo-control system can be fabricated directly on the disk, fabricated on a microchip and integrated into the disk or controlled through a system positioned external to the disk.

Detailed Description Text (88):
Fluorescence detector systems developed for macroscopic uses are known in the prior art and are adapted for use with the microsystem platforms of this invention. FIGS. 12A and 12B illustrate two representative fluorescence configurations. In FIG. 12A, an excitation source such as a laser is focused on an optically-transparent section of the disk. Light from any analytically-useful portion of the electromagnetic spectrum can be coupled with a disk material that is specifically transparent to light of a particular wavelength, permitting spectral properties of the light to be determined by the product or reagent occupying the reservoir interrogated by illumination with light. Alternatively, the selection of light at a particular wavelength can be paired with a material having geometries and refractive index properties resulting in total internal reflection of the illuminating light. This enables either detection of material on the surface of the disk through evanescent light propagation, or multiple reflections through the sample itself, which increases the path length considerably.

Detailed Description Text (121):
Sample or analyte is loaded onto the disk by the user. Sample is optimally loaded onto the disk at a position proximal to the center of rotation, thereby permitting the greatest amount of centripetal force to be applied to the sample, and providing the most extensive path across the surface of the disk, to maximize the number, length or arrangement of fluid-handling components available to interact with the sample. Multiple samples can be applied to the disk using a multiple loading device as shown in FIGS. 13A through 13C. In this embodiment of a multiple loading device, a multiplicity of pipette barrels are equally spaced and arranged radially. The pipettes are spaced to provide that the tips of the pipettes fit into access ports on the surface of the disk. The tips can be simple pins that hold a characteristic volume of sample by virtue of a combination of surface properties and fluid characteristics. Alternatively, the tips can be conventional hollow tubes, such as capillary or plastic conical tips, and the fluid manipulated manually in response to positive or negative pressure, as with a manual or automatic pipetting device. The loader can be operated manually or by robotic systems. The barrels can also be arrayed in a flexible arrangement, permitting the tips to address a linear array in one configuration and a radial array in another. In each embodiment, the loader comprises an alignment device to ensure reproducible placement of the loading tips on the disks of the invention.

Detailed Description Text (167):
The invention is advantageously used for microanalysis in research, especially biological research applications. Such microanalyses include immunoassay, in vitro amplification routines, including polymerase chain reaction, ligase chain reaction and magnetic chain reaction. Molecular and microbiological assays, including restriction enzyme digestion of DNA and DNA fragment size separation/fractionation can also be accomplished using the microsystem disks of the invention. Microsynthetic manipulations, such as DNA fragment ligation, replacement synthesis, radiolabeling and fluorescent or antigenic labeling can also be performed using the disks of the invention. Nucleic acid sequencing, using a variety of synthetic protocols using enzymatic replacement synthesis of DNA, can be performed, and resolution and analysis of the resulting nested set of single-stranded DNA fragments can be separated on the disk, identified and arranged into a sequence using resident software modified from such software currently available for macroscopic, automated DNA sequencing machines. Other applications include pH measurement, filtration and ultrafiltration, chromatography, including affinity chromatography and reverse-phase chromatography, electrophoresis, microbiological applications including microculture and identification of pathogens, flow cytometry, immunoassay and other heretofore conventional laboratory procedures performed at a macroscopic scale.

Detailed Description Text (201):
Exemplary of the uses the disks and devices of this aspect of the invention is the detection,, identification and size determination of DNA fragments produced by polymerase chain reaction or magnetic chain reaction (the latter disclosed in U.S. Ser. No. 08/375,226, filed Jan. 19, 1995, which is a file wrapper continuation of U.S. Ser. Nos. 08/074,345, filed Jun. 9, 1993 and 08/353,573, filed Dec. 8, 1994, each incorporated by reference in its entirety). Amplification is carried out using one primer labeled with a detectable label such as a fluorescent dye or radioisotope, and the other primer is covalently attached to a molecule that permits immobilization of the primer (e.g., biotin). After amplification (either off-disk or on the disk as described in more detail in Example 4 below), the labeled, biotinylated duplex DNA product fragment is attached to a solid support coated with streptavidin, for example, by movement of the amplification reaction mixture into a channel or compartment on the disk wherein the walls are coated with streptavidin, or by movement of the amplification mixture into a compartment on the disk containing a binding matrix such as Dynal M-280 Dynabeads (polystyrene coated paramagnetic particles of 2.8 um in diameter). Standardized size markers are included in the post-amplification compartment in order to provide a reference set of DNA fragments for comparison with the amplification product fragments. In this analysis, a number of different duplex DNA molecules from either a multiplex amplification reaction or a number of separate amplification reactions may be sized simultaneously, each fragment or set of fragments being distinguished from others by use of reaction- or fragment-specific detectable labels, or differences in some other physical property of the fragments. For amplifications performed off-disk, beads attached to the fragment are loaded into a channel on the disk capable of retaining the beads (such as size exclusion, "optical

tweezers" or by magnetic attraction). In the latter embodiment, the magnetic retention means (permanent magnets or electromagnets) are either integral to the disk, held on second disk spinning synchronously with the first, or placed on the device so as to immobilize the DNA fragments in the appropriate compartment.

Detailed Description Text (208):
Fragments of DNA are amplified in vitro by polymerase chain reaction (PCR) or magnetic chain reaction and analyzed by capillary electrophoresis. Reagent mixing, primer annealing, extension and denaturation in an amplification cycle resulting amplification of a 500 bp target fragment and its subsequent analysis are carried out using a device and disk as described in Example 1 above. A schematic diagram of the structure of the disk is shown in FIG. 21.

Detailed Description Text (215):
Amplified DNA fragments are analyzed by transfer to capillary electrophoresis unit H by spinning the disk at a speed of 1 to 30,000 rpm and opening a valve on reaction chamber G leading to capillary electrophoresis unit H, thereby effecting transfer of an amount of the reaction mixture to the electrophoresis unit. The amount of the reaction mixture, typically 10 .mu.L, is determined by a combination of the length of time the valve on reaction chamber G is open and the speed at which the disk is rotated. Capillary electrophoresis is accomplished as described below in Example 11, and fractionated DNA species detected using optical or other means as described above in Example 2. This method provides a unified amplification and analysis device advantageously used for performing PCR and other amplification reactions in a sample under conditions of limited sample.

Detailed Description Text (237):
After the exchange cycle is complete, the magnetic particles are transferred to a next reaction chamber containing 250 ug dry 1-ethyl-3(3-dimenthylaminopropyl) carbodiimide (EDAC). A reagent reservoir containing 170 OD (170 ng) 5'-aminated DNA oligonucleotide in 50 uL of 0.1 M imidazole solution chamber prior to addition of the particles in order to dissolve the EDAC. The particles are then added through a valve in about 100 uL 0.1 M imidazole. Upon addition of the magnetic particles to the reaction chamber, the device is stopped and incubated 6 hours at 40.degree. C. Heating can be effected by a heat source (such as Peltier heating device) embedded in the disk itself, or positioned in the instrument in a configuration permitting specific heating of the reaction chamber. In the latter alternative, the disk may be stopped at a predetermined position relative to the device to ensure specificity of heating of the reaction chamber.

Detailed Description Text (249):
Gel electrophoresis is performed on a disk prepared as described in Example 1 above. For this application, a gel media is prepared in the separation channel; however, such gel media must be protected from sheer forces that develop with rotation of the disk during transfer of sample or buffer to the electrophoresis channel. Thus, the gel-filled capillary is advantageously arrayed concentrically on the disk, as shown schematically in FIG. 29. As a result, the gel will only experience shear forces from centripetal-induced pressure during rotation if a fluid reservoir is in contact with the capillary during rotation of the disk. At rest, the planar geometry of the disk prevents hydrodynamic pressure on the capillary. This is an advantage over standard capillary electrophoresis systems, where hydrodynamic pressure is not so easily controlled because the buffer volumes and reservoir heights need to be carefully adjusted before each run to avoid hydrodynamic flow. This is also an advantage of capillary electrophoresis performed on the disks of the invention over electrophoresis performed on microchips, where buffer reservoirs are positioned above the plane of the separation channel and are thereby susceptible to hydrodynamic pressure-driven fluid flow. Gel electrophoresis is performed on the disks of the invention to separate DNA fragments, including duplex PCR fragments, oligonucleotides and single-stranded, dideoxynucleotide-terminated enzymatic DNA sequencing components, the system is configured as shown in FIG. 29. The disk is prepared comprising a polyacrylamide gel concentrically arrayed in a microetched separation channel in the disk The polyacrylamide gel is prepared from an unpolymerized solution of 7M urea, 45 mM Tris-borate buffer (pH 8.3), 1 mM EDTA, 9% acrylamide, 0.1% TEMED and 10% ammonium persulfate. The disk can be prepared in the separation channel by mixing the components (wherein it will be recognized that unpolymerized polymerized

polyacrylamide is susceptible to light-catalyzed polymerization upon storage) particularly by introducing TEMED and ammonium persulfate to the mixture. Sufficient gel mixture is added to the separation channel by opening a valve from a mixing chamber to the separation channel and rotating the disk at 1 to 30,000 rpm. The disk is stopped upon filing of the separation channel to permit gel polymerization. Shortly before polymerization is complete, the exit channel is flushed to eliminate bubbles and unpolymerized monomer by flushing the channel with buffer from a large buffer reservoir at the outlet side of the channel, controlled by a valve. A similar process is conducted on the inlet side of the gel.

Generate Collection

L8: Entry 1 of 5 File: USPT Apr 29, 2003

DOCUMENT-IDENTIFIER: US 6555389 B1
TITLE: Sample evaporative control

Detailed Description Text (9):
By having a network of channels, where some or all of the channels may interconnect, substantial flexibility is achieved. It is understood that for the purposes of this invention, channels and capillaries may be used interchangeably, where capillary intends that there is liquid movement upon introduction of liquid into one end of a channel due to surface tension. The channels may serve to deliver and remove agents from one or more zones, simultaneously or successively, depending on the plumbing employed. One may provide for miniaturized pumps, separation walls, gates, etc., so as to be able to direct liquids to specific zones. One may provide for successive replacement of liquids in the channels, whereby different reagents may be directed to the zones, which allows for modification of reactions, stepwise performance of reactions, removal of agents from the zones, etc. By modulating the temperature of the liquid in the channels one can modulate the temperature of the liquid in the zone. Thus, one could provide for heating and cooling of the mixture in the zone.

Detailed Description Text (13):
In this embodiment it appears that the evaporation from the zones results in the movement of liquid from the channel into the zone to retain the height of the meniscus. The liquid in the channel is, of course, maintained by the reservoir(s), whose volume will generally be large compared to the volume of the channel and the liquid in the zone. As a result of the meniscus in the zone, the evaporation from the zone per unit cross-sectional area of the zone will be greater than the evaporation per unit cross-sectional area of the reservoir. The difference may be further enhanced by having: a temperature differential between the liquid in the zone and the liquid in the reservoir; a differential air flow; a differential humidity; or the like, where the condition at the zone is to enhance the evaporation per unit area at the zone, as compared to the reservoir. The temperature during the time of addition may be ambient, reduced or elevated, generally in the range of about 10.degree. C. to about 65.degree. C., more usually in the range of about 20.degree. C. to 50.degree. C., so long as the rate of evaporation is not unduly great to interfere with the replenishment.

Detailed Description Text (25):
Capillary channels would lead from the dilution vessel to one or more, usually a plurality of zones, where the diluted sample would migrate by capillary action to the individual zones. As appropriate, pneumatics, including a hydrostatic head, may be used to direct the flow of the liquids. The liquid from the dilution vessel would mix with other liquid(s) in the zone. In this way, small volumes of a reagent or candidate compound would be distributed among a number of zones for a subsequent operation, without initially having to manipulate small volumes. The same mechanism may be used to distribute an expensive reagent to a plurality of zones. In this situation, it may not be necessary to dilute the reagent, but the reagent may be directly added to the central vessel. The reagent would then be distributed from the vessel to the various zones. Desirably, the capillary channels will be relatively short, usually less than 1 cm, more usually less than about 0.5 cm and more than about 0.1 nm. The volume of the vessel will usually be at least 100 nl, more usually at least about 300 nl and less than about 1 ml, usually less than about 0.5 ml, depending on the amount of the solution to be transferred to each of the zones and the number of zones. By having a central vessel for distribution to a plurality of zones, one can reduce errors in transferring small volumes and provide for substantially equivalent transfer to a plurality of zones, allowing for direct comparison of results in each of the zones.

Detailed Description Text (38):
In order to automatically determine when the desired liquid volume has been introduced

into the zone, rather than relying on the parameters which were used to pump the liquid into the zone, such as voltage, time, temperature, etc., one can provide for a detection system. One system uses an ionic medium, conveniently introduced into a channel connected to the zone, with a detection electrode in the ionic medium connected to a voltage source or ground. When electrokinetic pumping is employed, there will be an electrical field in the fluid. When the fluid in the zone contacts the ionic medium, a circuit will be formed with the detection electrode, which can be detected and further pumping terminated or the electrical field will be grounded and further pumping stopped. One may simply have an electrode in the zone, which when contacted with the liquid from the channel will act as described above. Instead of an electrical detection system, one may use an optical system, which detects the extent to which the liquid has penetrated the zone. The particular mode of detection will depend to some degree on the choice of the mode of transferring the fluid into and out of the zone.

Detailed Description Text (44):
The size of the zone will be affected by the sizes of the ports, outlets and channels, volumes of the solutions added to the zones, the amount of liquid in the channel into which the components of the added solutions diffuse, by the nature (regions of wettability and non-wettability) of the walls enclosing the zone, the rate of evaporation, which may be related to the humidity, depth of the zone and air flow above the zone, the time of the reaction, the temperature, the composition of the solution in the channel, particularly as to the solution viscosity, and the like. Generally, these parameters will be selected to provide a dilution in the zone of the sample component added to the zone in the range of about 0.1 to 10:1, during the course of the reaction. Incubations may involve from about 1 min. to 24 h, usually not exceeding about 12 h. The reaction time will usually require at least 1 min., usually at lest about 5 mins, and not more than about 6 h, usually not more than abut 2 h. Ambient conditions will usually suffice, with temperatures below about 60.degree. C., more usually not more than about 40.degree. C. In some situations where thermal cycling is involved, temperatures may be as high as 95.degree. C., usually not exceeding about 85.degree. C., and cycling between 45.degree. C. and 95.degree. C. Heating can be achieved with lasers, light flashes, resistance heaters, infrared, heat transfer, conduction, magnetic heaters, and the like.

Detailed Description Text (61):
The device may provide for heating and cooling of the zone. By varying the temperature of the channel, a large heat sink or source is provided for the zone. By having means for heating or cooling the fluid in the channel, one can modify the temperature of the zone, cycling the zone temperature in relation to the channel. To provide for more rapid variation in temperature, one may provide for heating and/or cooling solely in the zone, where once the source of thermal variation in the zone is terminated, the zone would rapidly equilibrate with the temperature of the channel. For example, in thermal cycling, one could use microwave heating, RF heating, laser heating, or the like, where the electromagnetic heating source is focused on the zone, so as primarily to change the temperature of the zone. In processes involving thermal cycling, such as the polymerase chain reaction, one would rapidly raise the temperature of the zone to 85-95.degree. C., while maintaining the channel temperature at about 35-50.degree. C. Once the DNA has been denatured, which would be a matter of not more than about 2 or 3 minutes, usually less, by removing the source of heat, the liquid in the zone would rapidly equilibrate with the temperature of the liquid in the channel. By appropriate selection of the temperature of the liquid in the channel, the temperature profile during the cycling may be controlled to provide the desired times for the different temperature stages of the cycle.

End of Result Set

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L4: Entry 1 of 1 File: USPT Jan 5, 1993

DOCUMENT-IDENTIFIER: US 5176203 A
TITLE: Apparatus for repeated automatic execution of a thermal cycle for treatment of samples

Abstract Text (1):
Apparatus for repeated automatic execution of a thermal cycle for the treatment of a sample, especially a biological sample, comprises means (6) defining a pathway which is physically closed throughout the treatment and within which the sample is resident throughout the treatment. Means (3, 4, 11, 12) are provided to move the sample between different positions along the pathway and (7, 15, 16) to heat or cool the sample as a function of its position within the pathway. Preferably the pathway is a capillary tube, which may be in spiral, closed loop or linear form. Heating and cooling means are provided by thermostated zones which may provide a continuous thermal gradient regular or irregular, or be in a discontinuous arrangement. The preferred means of moving the samples is magnetic.

Brief Summary Text (3):
The Tm of a DNA sequence depends essentially on the following two parameters: the basic sequence and ionic force of the medium. The Tm usually found varies between 20.degree. and 85.degree. C. Thus, the great majority of molecular reactions may be produced in perfectly defined and controlled thermal conditions. Certain of these reactions require the successive use of different temperatures and may be effected in the apparatus described in this invention. This particularly concerns hydrolysis using restriction enzymes, enzyme modification reactions for DNA, cascade enzyme reactions, the isolation of repetitive families of sequences for DNA and the amplification by `polymerase chain reaction`. These applications will now be discussed in greater detail.

Brief Summary Text (17):
Amplification of the number of DNA sequences by `Polymerase chain reaction` (PCR)

Brief Summary Text (18):
This technique enables one specifically to amplify the number of copies of a double strand DNA sequence. The principle of PCR (R. K. Saiki et al, Science, 230, 1985, 1350-1354) is to use the activity of the DNA polymerase DNA dependent initiating the synthesis starting from oligonucleotidic initial material (P1 and P2) added in the reaction medium. An amplification cycle consists of three successive stages:

Brief Summary Text (26):
Thus, after an amplification cycle, the number of sequences completed by P1 and P2 is multiplied by 2, multiplied by 4 after 2 cycles, by 8 after 3 cycles, by 1024 after 10 cycles and by 1,048,576 after 20 cycles. Generally, the rate of amplification after n cycles is 2.sup.n. A cycle of amplification thus consists of 3 successive thermal stages and one complete PCR reaction requires about 10 to 60 cycles. Each thermal stage generally lasts from 1 to 5 mins. Automation of such a technique thus represents considerable progress.

Brief Summary Text (29):
Various spatial arrangements of the capillary tube are envisaged. It may, for instance, be in a spiral form, in a closed loop form or in linear form. Each turn of the spiral, each turn of the closed loop or each passage of the length of linear capillary tube represents one thermal cycle, within which the sample passes through two or more thermostated zones, at different temperatures from 4.degree. C. to 150.degree. C. Further thermal cycles, up to 100, comprise the next turn of spiral, a

further turn within the closed loop, or the return of the sample in reverse direction along the length of the linear capillary tube.

Brief Summary Text (31):
The rate of movement of the sample through the capillary tube has a profound effect on the treatment. If the sample moves very slowly through a zone, its temperature will approach or reach the zone temperature. The sample may even be stopped within a given zone for a predetermined period, stabilising its temperature at that of the zone. On the other hand, if the sample moves rapidly through a zone, the thermal effect of that zone on the sample may be minimised or suppressed.

CLAIMS:

1. Apparatus for the treatment of biological and microbiological samples comprising:

(a) a pathway which is physically closed throughout the treatment;

(b) means for introducing one of said biological or microbiological samples to the pathway prior to the commencement of treatment and for removing said sample subsequent to the completion of treatment;

(c) means for physically closing the pathway during treatment so that nothing can be added thereto or withdrawn therefrom;

(d) a plurality of thermal means positioned along the pathway for heating or cooling of said sample, said thermal means providing treatment of said sample at a temperature of from 4.degree. C. to 150.degree. C.; and

(e) means for automatically and repeatedly, through a plurality of cycles, moving said sample between said different thermal positions along the pathway.

12. Apparatus according to claim 1 in which the means for moving the sample comprises magnetic means including magnetic party within the pathway adjacent to the sample and an external magnet acting on the magnetic party to move it and hence the sample.

18. Apparatus for the treatment of biological and microbiological samples comprising:

(a) a loop pathway which is physically closed throughout the treatment;

(b) means for introducing one of said biological or microbiological samples to the loop pathway prior to the commencement of treatment and for removing said sample subsequent to the completion of treatment;

(c) means for physically closing the loop pathway during treatment so that nothing can be added thereto or withdrawn therefrom;

(d) a plurality of thermal means positioned along the loop pathway for heating or cooling of said sample, said thermal means providing treatment of said sample at a temperature of from 4.degree. C. to 150.degree. C.; and

(e) means for automatically and repeatedly, through a plurality of cycles, moving said sample between said different thermal positions along the loop pathway.

End of Result Set

☐ Generate Collection

L4: Entry 1 of 1 File: USPT Jul 2, 2002

DOCUMENT-IDENTIFIER: US 6413780 B1
TITLE: Structure and method for performing a determination of an item of interest in a sample

US Patent No. (1):
6413780

Detailed Description Text (12):
Magnet 4 can be moved with respect to the first container 1 at selected times during performance of a given determination of an item of interest in a sample in the first container 1. The movement of the magnet 4 can effect performance of a step in the determination process thereby allowing that step to be selectively automatically performed or avoided as desired. In one embodiment, the magnet 4 may be moved relatively proximate to the container to attract magnetically responsive particles within the first container 1 to a side wall of first container 1 thereby separating those particles which may be bound with a desired item of interest in a patient sample from the remaining patient sample or other contents of the first container 1.

Detailed Description Text (13):
Before, during or after such magnet 4 induced separation, probe 3 may aspirate a portion of the first container 1 contents to waste/wash reservoir 10. Subsequent dispense, separation, and aspiration steps may be employed to enhance the item of interest determination. During periods of the determination where magnetic separation is not desired, i.e. the magnetic separation step is avoided, magnet 4 may be moved relatively distally with respect to the first container 1 to reduce effects of the magnetic field of the magnet 4 on the first container 1 and its contents. If desired, magnetically responsive particles to which no item of interest is attached may be attracted to the side wall of the first container 1 while the remaining contents, possible containing an item of interest, of the first container 1 is removed from first container 1, such as by the probe 3.

Detailed Description Text (15):
At various times during performance of a given determination of an item of interest, a sample disposed in container 8 and reagent contained in container 9 may be added to first container 1, such as by probe 3. If multiple samples and/or reagents are desired, an array, such as a conveyor, a carousel, other movable arrangement, possibly recirculating, or the like, of multiple containers 8 and/or 9 could be provided. Containers 8 and 9 could be fabricated out of any suitable material, such as a polymer like polystyrene (DOW 666), high-density polyethylene (DOW 30460M HDPE or Chevron 9512) respectively, and the like.

Detailed Description Text (35):
After engagement of a tip 28 by either pipettor 19 or 12, liquid level sensing (executed by any currently available method), aspiration from selected container(s), and dispense to first container 1 occurs. Pipettor 12 or 19 may include an apparatus which can detect a liquid level and/or temperature. This apparatus may include, but is not limited to, photo optics, capacitive members, IR, sonar, or other wave form generators. After dispense, tip 28 is washed with liquid at wash station 23 thereby reducing exposure to a contaminant. Subsequent additions to first container 1 may occur in similar fashion, as desired. After all desired additions to first container 1 have been completed, first container 1 contents may be is aspirated or otherwise removed from first container 1 and dispensed or transferred to desired locations where other functions, such as genetic sequencing, a pharmacogenetic test and the like, can be performed. Then, the tip 28 may be removed from pipettor 12 or 19 and disposed to

tip 28 waste 24, thereby reducing exposure to a contaminant. By using a single tip 28 for multiple reagent and singular sample or prepared sample manipulations can reduce solid waste and can provide reduced cost while maintaining desired levels of contamination reduction. Similar steps may be performed with the pipettors 12 or 19 even if they do not include a tip 28.

Detailed Description Text (67):
FIG. 22 shows another structure 1e where modules 16b can be duplicated according to sample sorting outcomes. FIGS. 23 and 24 show other structures 1f and 1g where modules 16 can be located exterior to the structure(s). Here, sorted samples can be duplicated across multiple modules 16 exterior to the structure(s) 1f and 1g.

Detailed Description Text (134):
At position 76, pipettor 12 engages a disposable pipettor tip 28, aspirates a first reagent from a container in reagent storage area 13, and dispenses that reagent into second container 15 on container processor line 15a. The disposable pipette tip 28 is washed with fluid in wash cup 24. Pipettor 12 aspirates a second reagent from a container in reagent handling area 13, dispenses the second reagent into second container 15, and disposable pipette tip 28 is washed with in wash cup 24. A third reagent is aspirated into pipette tip 28 from a container in reagent handling area 13, and the first container 1 contents containing the item of interest, about 50 .mu.L, is aspirated from first container 1 in position 77 of first process path 11 to the pipette tip 28. The third reagent and the aspirated first container 1 contents are dispensed from the pipette tip 28 into second container 15 and pipettor 12 ejects disposable pipette tip 28 to tip waste 24. Alternately, the third reagent can be dispensed into first container 1 on first process path 11 at position 76 by pipettor 12 or by another dispense nozzle on the first process path 11. In another embodiment, the first reagent and second reagent aspirations can be completed, without washing the pipettor 12 between aspirations, and the reagents can be dispensed into second container 15 substantially simultaneously. The volumes of each of the three reagents may be substantially within the range of about 10 to about 50 .mu.L. If it were desired to detect more than one item of interest in a given sample, portions of the contents of first container 1 can be transferred to a corresponding number of containers 15. These multiple transfers of first container 1 contents may occur from position 77 or, alternately, may occur from position 77 and subsequent position(s). If a relatively large number, such as about 15, of items of interest are to be determined from one sample, then multiple aspirations and dispenses can occur from container 8 and/or first container 1 by pipettors 19 and/or 12.